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**Driving factors of soil microbial ecology in alpine, mid-latitude patterned grounds (NW Italian Alps)**

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*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1603097> since 2019-12-19T17:49:56Z

*Published version:*

DOI:10.1007/s00374-016-1147-z

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## Abstract:

Patterned ground (PG) is one of the most evident expressions of cryogenic processes affecting periglacial soils, where macroscopic, repeated variations in soil morphology seem to be associated with small-scale edaphic and vegetation gradients, potentially influencing also microbial communities. While for high latitude environments only few studies on PG microbiology are available, the alpine context, where PG features are rarer, is almost unexplored under this point of view. We followed a double approach, based on Denaturing Gradient Gel Electrophoresis (DGGE) and quantitative PCR (qPCR), in order to investigate microbial community composition and ~~to-evaluate the~~ abundance of phylogenetic markers and functional genes (bacterial and archaeal *amoA*) within single PG features and among different sites from four areas in the Western Italian Alps, characterized by different lithotypes. Bacterial, archaeal and fungal community composition was quite homogeneous within single features, with more differences among samples collected from different lithologies. The abundance of phylogenetic and functional markers was uniform at different sites, except for the highest altitude one showing the lowest bacterial, archaeal and ammonia-oxidizing archaea abundance. Nevertheless, at a small-scale level, a concentric distribution of microbial markers was described within single features, paralleling soil chemical properties trends. These first results support the hypothesis that microbial ecology in alpine, periglacial ecosystems is driven by a complex series of environmental factors, such as lithology, altitude and cryogenic activity, acting simultaneously on community shaping both in terms of diversity and abundance.

## Keywords:

Periglacial alpine soils; Cryoturbation; Lithology; Community structure; Microbial abundance

## Introduction

Patterned ground (PG) derives from cryogenic processes and represents one of the most spectacular expression in periglacial landscapes. Cyclic soil freezing and thawing, accompanied by ice lens formation, leads to severe ground modifications, resulting in surface geometric patterns including circles, polygons, networks or stripes. The presence of textural sorting, with stony areas alternated to soil and fine debris characterizes *sorted* patterned ground, while in *nonsorted* patterned ground single features are defined by differences in ground relief or vegetation cover. In both cases, two domains are often recognizable: central parts of finer material or bare ground, more strongly affected by cryogenic processes; and peripheral areas, richer in stones and/or vegetation (Ballantyne et al. 2013; Walker et al. 2008). These macroscopic, repeated variations in ground morphology produce also small-scale gradients in physical and chemical soil properties, changing between centres and rims (Barrett et al. 2004; D'Amico et al. 2015; Michaelson et al. 2012; Wagner et al. 2005). In this sense, single patterned ground features can be seen as small, ubiquitous model unities useful to investigate the effect of cryoturbation processes on soil evolution, plant colonization, and organic C accumulation and storage in geographically, climatically and topographically diverse environments. In fact, patterned ground formation is widespread in high-latitude environments, such as polar or sub-polar regions, but occurs also in alpine areas, in presence of permafrost or seasonal ground freezing conditions and favourable topographic conditions (Ballantyne et al. 2013).

Until now, several works recognized the presence of a strong interaction between soil processes and vegetation in patterned ground formation and functioning (D'Amico et al. 2015; Michaelson et al. 2012; Walker et al. 2008), but the effects on soil microbial properties are poorly known. Microorganisms are able to survive, grow and be metabolically active in very harsh conditions, such as subzero temperatures, presence of ice and freeze-thaw cycles (Margesin and Miteva 2011; Steven et al. 2006). Moreover, they play key roles in weathering processes, pedogenesis, biogeochemical cycling and plant colonization of permafrost soils and recently deglaciated areas, like glacier forefields (Bajerski and Wagner 2013; Jansson and Taş 2014; Nemerut et al. 2006). Considering the impact of microbial communities on soil

ecosystem properties, it is necessary to increase our comprehension of the role of microbial communities in a complex and dynamic pedo-environment like patterned ground.

To date, only a limited number of studies considered patterned grounds from a microbial point of view, all referring to Arctic or Antarctic areas. In particular, several studies focused on polygonal soils, due to their large diffusion in tundra ecosystems and to their potential role in global methane production linked to the waterlogged, anoxic conditions affecting these soils. Differences in terms of community composition were described along depth gradients (Frank-Fahle et al. 2014; Wagner et al. 2005), comparing central and marginal areas of single features (Wagner et al. 2005), and considering different polygonal soils (Frank-Fahle et al. 2014; Lawley et al. 2004). Another line of investigation concerned patterned grounds along the North American Arctic Transect. Timling et al. (2014) and González et al. (2014) compared microbial communities in terms of biomass and fungal community composition in patterned ground features (PGF) and adjacent vegetated soils (AVS) along a topographic and climatic gradient, detecting significant differences between PGF and AVS both in terms of microbial biomass and diversity, coherently with differential distribution of plant cover and soil properties already described (Walker et al. 2008, 2011). On a broader scale, they noticed that the hierarchy of environmental factors potentially involved in community shaping changes within the bioclimatic gradient. For instance, in more extreme environments, such as the higher latitude polar deserts, they found that disturbances linked to cryoturbation have smaller impacts on microbial biomass and community composition than at lower latitudes, resulting in limited differences between PGF and AVS. Conversely, topographic position, analyzed by González et al. (2014) by comparing dry, wet and mesic zones, becomes a more important driver in warmer subzones.

While patterned ground landscapes are extensively distributed in Arctic and subarctic regions, in mid-latitude mountain ranges their diffusion is limited to few areas characterized by flat surfaces, rapid snow removal by wind and high water availability (Bockheim and Munroe 2014). For this reason, the identification of sites suitable for the study of the combined action of climatic conditions and cryoturbation disturbances on soil microbial communities is rather complex. On the other hand, the presence of the same phenomenon replicated in sites geographically close to each other gives the opportunity to investigate the influence of other environmental drivers potentially involved in communities and ecosystem modelling, like altitude, parent material lithology and soil properties. The parent material

lithology, in particular, has a strong impact not only on the morphology of patterned ground features, due to the different resistance to ice-driven weathering, but also on soil chemical properties and plant colonization (Michaelson et al. 2008). This suggests that also the composition and distribution of microbial communities might be influenced by these properties, as already reported for other cold ecosystems (Boyd et al. 2007; Larouche et al. 2012; Nyssönen et al. 2014; Reith et al. 2015).

With this study, we performed a preliminary investigation –the first, on our best knowledge– on microbial communities inhabiting patterned ground features in an alpine context, in terms of composition, overall diversity and abundance. We chose four active patterned ground sites in the North-Western Italian Alps, developed on different lithotypes creating large gradients in chemical soil properties such as available nutrients and heavy metal contents. Our hypothesis was that, as for chemical soil properties and plant distribution, cryoturbation should have an impact also on microbial population, both in terms of biomass distribution and community composition. The intensity of this influence should be modulated by site-specific edaphic properties linked to parent material lithology. This work had thus three main objectives: 1) to give a first insight in the microbial ecology of a fascinating and previously almost unexplored ecosystem; 2) to describe and compare microbial diversity and distribution both at a small-scale, within single PG features, and among different sites; 3) to define soil properties potentially involved in shaping microbial communities composition.

## Materials and methods

### Sample collection

For this study, four active patterned ground areas, located in the Western Italian Alps and dominated by stripes, sorted and nonsorted circles, were chosen. All areas were located in protected areas (Mont Avic Natural Park and Gran Paradiso National Park).

The different sites were characterized by different parent material (Table 1):

- caeschists (CS site), serpentinite and metamorphic gabbros respectively in SP and GB sites, and frost shattered gneiss at GN site. In CS and GB, the parent materials were enriched in small quantities of serpentinite derived from upslope areas.

For each site, one typical PG feature was examined in order to minimize the sampling impact on these ecosystems. Five surface samples (0-10 cm) were collected equally spaced along a north-south transect drawn across the circle/stripes. Hence, we obtained two external

1 samples, taken from the stony/vegetated rims (N and S), one central sample (C) and two  
2 intermediate samples (NC and SC), as shown in Fig. 1. A total of 20 samples was obtained.  
3 Sampling took place in late September 2012. In that period, nighttime air temperatures  
4 were expected to drop below freezing point, enhancing freeze-thaw cycling; below the  
5 sampled depth, in fact, the soils were completely frozen. All the samples were collected in the  
6 early afternoon, stored at 4 °C in the field and during the transport and at – 20 °C in the  
7 laboratory prior to further analysis.

8 Climatic conditions of the study areas and morphological, mineralogical and textural  
9 characteristics of PG soils, as well as vegetation type and distribution, are described in detail  
10 in D'Amico et al. 2015. Soil chemical properties and percentage of vegetation cover,  
11 measured at each sampling point and reported in the same study, are summarized in Table 2.

12

13 **Soil DNA extraction**

14 Total soil DNA was extracted from 0.5 g of soil samples using the FastDNA™ SPIN Kit for  
15 Soil and the FastPrep® Instruments (MP Biomedicals) in accordance with the manufacturer's  
16 instructions. Quantity, quality and integrity of extracted DNA were evaluated using a  
17 NanoDrop ND-1000 spectrophotometer (Thermo Fischer Scientific) and agarose gel  
18 electrophoresis.

19

20 **PCR-DGGE**

21 Polymerase chain reaction (PCR) products for denaturing gradient gel electrophoresis  
22 (DGGE) were obtained by amplifying total bacterial and archaeal 16S rRNA genes and fungal  
23 26S rRNA genes. While bacterial and fungal genes were amplified directly from the extracted  
24 DNA, a nested approach was followed for Archaea. Primer pairs were 357F-GC and 518R-  
25 ~~(Muyzer et al. 1993)~~ for bacteria ~~(Muyzer et al. 1993)~~, NL1 and LS2 ~~(O'Donnell 1993)~~;  
26 ~~Coccolin et al. 2000)~~ for fungi ~~(O'Donnell 1993; Coccolin et al. 2000)~~, A2F and 1492R-  
27 ~~(Reysenbaech et al. 1995; Lane 1994)~~ and SaF-GC and PARCH519R ~~(Nieol et al. 2003)~~;  
28 ~~Øvreås et al. 1997)~~ for the first and second step of archaeal PCR respectively ~~(Reysenbaech et~~  
29 ~~al. 1995; Lane 1991; O'Donnell 1993; Coccolin et al. 2000)~~. Primer sequences and reaction  
30 conditions are reported in Online Resource 2.

31 All PCR reactions were carried out in a DNAEngine® Peltier Thermal Cycler (Bio-  
32 Rad Laboratories) in a 25 µl reaction volume containing 1 x reaction buffer (Bioline), 3 mM

1 MgCl<sub>2</sub>, 0.02 bovine serum albumin (BSA), 0.2 mM of each dNTP, 0.4 µM of each primer,  
2 1.25 U of BIOTAQ™ DNA polymerase (Bioline) and 2 µl of soil DNA diluted 1:10 in sterile  
3 DNase-treated water (Sigma). Second steps of nested PCR were performed without BSA,  
4 using 1 µl of the first step product as template.

5 DGGE was carried out as previously described by Webster et al. (2006) using a  
6 DCode™ Universal Mutation Detection System (Bio-Rad Laboratories), with a gradient from  
7 30 to 60%. Electrophoresis was run at 200 V for 5 h (with an initial 10 min at 80 V) at 60°C  
8 in 1 x TAE buffer. Gels were stained for 30 min with SYBR® Gold nucleic acid gel stain  
9 (Invitrogen) and visualized under UV with an UVpro Platinum Gel Documentation System  
10 (UVItec).

11 Reproducibility of DGGE profiles was tested by comparing PCR products obtained by  
12 using DNA extracted in triplicate from the same sample as template. Considering that good  
13 reproducibility was achieved, DGGE gels were organized in order to compare single samples  
14 within PG features and among different sites (Online Resource 1). DGGE bands recurrent at  
15 site level, or shared among different sites were excised, incubated one night at -20°C, washed  
16 and crushed in 10-20 µl of molecular-grade water. Supernatant (1 µl) was used as template  
17 and PCR was performed as above except for the elimination of BSA and the employment of  
18 modified linker-PCR archaeal and bacterial primers described in O'Sullivan (2008). PCR  
19 products were sequenced and searched for sequence similarities in the National Center for  
20 Biotechnology Information database using nucleotide Basic Local Alignment Search Tool  
21 (BLASTn) analysis (Altschul 1990).

22 Obtained 16S bacterial rRNA gene sequences were submitted to the [European](#)  
23 [Nucleotide Archive/EMBL database](#) (<http://www.ebi.ac.uk/ena>) under [accession numbers](#):-  
24 [Submission code was Hx20000054952](#). LT613607-LT613635.

25

26 **Quantitative PCR**

27 The abundance of different phylogenetic markers and functional genes was estimated by real-  
28 time quantitative PCR (qPCR).

29 For standard curves construction, the reference genes were amplified from genomic  
30 DNA extracted from pure cultures of standard organisms: *Lactococcus lactis* subsp. *cremoris*  
31 for bacterial 16S, *Methanococcoides methylans* for archaeal 16S, *Saccharomyces cerevisiae*  
32 for eukaryotic 26S and *Nitrosomonas europaea* for bacterial *amoA*. PCR products were than

1 purified with the PCRExtract Mini Kit (5 Prime), in accordance with the manufacturer's  
 2 instructions, quantified by NanoDrop and used to prepare serial dilutions in molecular-grade  
 3 water.

4 Primer pairs used for standard preparation were 27F and 1492R (Lane 1991) for  
 5 bacteria (Lane 1991), S-D-Arch-0025-a-S-17 and 1517R (Vetriani et al. 1999) for archaea  
 6 (Vetriani et al. 1999), NL1 and LS2 (O'Donnell 1993; Coccolin et al. 2000) for fungi  
 7 (O'Donnell 1993; Coccolin et al. 2000) and amoA-1F and amoA-2R (McTavish et al. 1993)  
 8 for bacterial *amoA* genes (McTavish et al. 1993). Primer sequences and references for PCR  
 9 conditions are reported in Online Resource 3, while master mix composition was as described  
 10 above (excluding BSA).

11 Only for archaeal amoA gene, PCR products obtained by amplifying total DNA  
 12 extracted from PG sample 2N with primer pair Arch-amoAF and Arch-amoAR (Francis et al.  
 13 2005) were pooled, purified, quantified by NanoDrop, serially diluted and used for standard  
 14 curve construction.

15 qPCR reactions were performed using a Chromo4™ Real Time PCR Detection  
 16 System (Bio-Rad Laboratories), and data were analysed with the MJ Opticon Monitor  
 17 software (version 3.1). Primer pairs were the same as for standard preparation, except for  
 18 Bacteria and Archaea. The first were substituted by the pair 519F and 907R (Lane 1991;  
 19 Muyzer et al. 1995), the second by the pair S-D-Arch-0025-a-S-17 and S-D-Arch-0344-a-S-  
 20 20 (Vetriani et al. 1999). The PCR mixture contained 0.3 µM of each primer, 10 µl of  
 21 SsoAdvanced™ SYBR® Green Supremix and 2 µl of soil DNA diluted 1:10 - 1:100, in a total  
 22 volume of 20 µl. Optimal DNA dilution was chosen in order to minimize inhibition problems  
 23 linked to low A260/A230 ratio of extracted DNA. All the samples and the standards were  
 24 analysed in triplicate on PCR strip tubes (Bio-Rad Laboratories) with the following thermal  
 25 cycling conditions: 95° for 5 min, followed by 40 cycles of 95°C for 30 sec, annealing for 30  
 26 sec and 72°C for 1 min. Different annealing temperatures are reported in Online Resource 3.  
 27 PCR specificity was verified by melting curves analysis. Standard curves R<sup>2</sup> value was always  
 28 higher than 0.996, and all the reactions showed efficiencies higher than 70%.

29

30 **Statistical analysis**

31 Statistical analysis was performed using R 3.0.1 software (R Foundation for Statistical  
 32 Software, Institute for Statistics and Mathematics, Vienna, Austria).

1 Significant differences in gene abundance among different lithologies were checked  
 2 by Brown-Forsythe one-way ANOVA combined with post hoc Games-Howell test and  
 3 displayed as boxplots, using the userfriendlyscience package.

4 Microbial communities were grouped using Cluster Analysis (CA), average linkage  
 5 agglomeration criteria, Bray-Curtis dissimilarity algorithm. The best dissimilarity algorithm  
 6 (Bray-Curtis) was selected according to the function rank index in the Vegan package  
 7 (Oksanen et al. 2013), which correlates many dissimilarity algorithms with a given gradient  
 8 (in this case, soil-environmental properties). As the clusters were usually very well separated,  
 9 their statistical significance was not checked.

10 Gradients in microbial community composition within the different patterned ground  
 11 sites were observed using unconstrained ordination methods (NMDS, Kruskal, 1964, distance  
 12 Bray-Curtis). The analysis was carried out with metaMDS within R vegan, using a Wisconsin  
 13 double standardization and a maximum number of 100 runs to reach the best solution (two  
 14 axis). To visualize relationships between microbial community composition and  
 15 environmental parameters, the resulting NMDS biplot was interpreted using a post-hoc  
 16 correlation with significant soil and environmental parameters (function envfit).

17 Pearson's linear correlation coefficients were calculated for assessing significant  
 18 relations between microbial abundance and environmental parameters.

19

20 **Results**

21 **Community structure (PCR-DGGE)**

22 Bacterial DGGE profiles showed a quite homogeneous community composition within single  
 23 PG features, with more differences among the four sites (Online Resource 1). A pool of  
 24 ubiquitous, brightly stained bands was clearly recognizable beside several site-specific, often  
 25 weaker bands. Sequence analysis of excised bands highlighted the presence of at least 7  
 26 different phyla (*Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*,  
 27 *Bacteroidetes*, *Cyanobacteria*, and *Chloroflexi*), with all the ubiquitous phylotypes belonging  
 28 to *Acidobacteria* or *Alphaproteobacteria* (Table 3). Only two of the detected bacterial  
 29 phylotypes detected were closely related to described bacterial species (>97% sequence  
 30 similarity), while the majority was related to uncultured bacteria previously found in soils or  
 31 periglacial ecosystems. Cluster analysis confirmed the presence of distinct bacterial  
 32 community composition at all the sites (Fig. 2).



1 Archaeal DGGE profiles appeared more heterogeneous compared to Bacteria at a  
2 small-scale level, both in terms of bands number and intensity, and few dominant bands  
3 recurred in samples collected from different sites (Online Resource 1). Archaeal community  
4 [structure](#) [composition](#) in PG sites separated in different groups, as indicated by cluster  
5 analysis. Only at SP site a separation between centre and rims was detected (Fig. 2). All the  
6 sequences obtained from excised bands belonged to *Thaumarchaeota*, and showed 93-96% of  
7 sequence similarity with *Candidatus Nitrososphaera gargensis* or *Candidatus Nitrososphaera*  
8 *evergladensis*. Nearly all the phylotypes were closely related to uncultured Archaea  
9 previously detected in high-altitude soils (as reported in Table 4) but also with DNA  
10 sequences retrieved from temperate agricultural and forest soils.

11 Our PCR-DGGE approach was able to detect only low fungal diversity. As for  
12 Bacteria, the main differences among profiles seemed to be linked to the site, rather than the  
13 position within PG features. Cluster analysis supported this interpretation, even if for GB and  
14 GN samples a clear separation has not been found. Sample 3N was excluded from the analysis  
15 due to the impossibility to obtain a clear DGGE profile (Fig. 2). Only few sequences were  
16 obtained from fungal excised bands, including bands recurrent at site level, or shared among  
17 different sites, all corresponding to *Ascomycota* or *Basidiomycota* (Table 5); interestingly,  
18 four of them were strictly related (97-98% similarity) to uncultured *Ascomycota* detected in  
19 PGs from North American Arctic by Timling et al. (2014).

## 21 Microbial abundance

22 Microbial abundances were assessed by quantifying bacterial and archaeal 16S rRNA genes  
23 and fungal 26S genes with a qPCR-based method. Abundance of ammonia oxidizers was  
24 estimated by quantifying bacterial and archaeal ammonia monooxygenase subunit A (*amoA*)  
25 genes. Also total DNA concentration was considered, as a proxy for biotic presence.

26 Comparing samples from different lithologies, significant differences ( $P < 0.05$ ) were  
27 only found for DNA, bacterial and archaeal markers and for archaeal *amoA* genes (Fig. 3). In  
28 general, lower abundances were reported for GN samples (average of 9.10, 6.31 and 6.67 Log  
29 copies per g of dry soil for Bacteria, Archaea and AOA respectively), while CS, SP and GB  
30 showed similar values (average of 9.92-10.12, 6.69-7.57 and 7.21-7.87 Log copies per g of  
31 dry soil for Bacteria, Archaea and AOA respectively). Fungal marker abundance ranged from  
32 an average of 8.57 to 9.27 Log copies per g of dry soil, without significant differences among

1 sites. Neither for bacterial *amoA* genes abundance, ranging from an average of 4.34 to 5.59  
2 Log copies per g of dry soil, significant differences were detected. Comparing only functional  
3 genes, a predominance of archaeal over bacterial *amoA* genes, with AOA/AOB Log copies  
4 ratio ranging from 1.4 to 1.9, was detected in all the four sites.

5 At a small-scale level, a slightly concentric variation was reported for all the  
6 phylogenetic markers: the abundance of bacterial and archaeal genes decreasing from the rims  
7 toward the centre of single features in CS, GB and GN, and showing an opposite trend in SP;  
8 fungal markers reaching the highest values in intermediate positions (Fig. 4). Also DNA  
9 concentration followed a similar trend, decreasing from the rims to the centre in CS, GB and  
10 GN and from the centre to the rims in SP. No clear repeated patterns were described observing  
11 the distribution of AOA and AOB markers within single PG features. The only recognizable  
12 trends were a concentric decreasing of AOB in GB and AOA in GN or the north-to-south  
13 decreasing of AOB in GN and AOA in SP.

## 15 Discussion

16 We performed a preliminary survey, in order to explore the composition and abundance of mi-  
17 crobial communities inhabiting patterned ground features developed on lithologically distinct  
18 sites in a mid-latitude alpine environment. A molecular approach combining PCR-DGGE  
19 (community structure analysis) and qPCR (quantitative analysis) was applied, targeting bacte-  
20 rial, archaeal, and fungal phylogenetic markers. Moreover, in order to focus on microbial  
21 driven processes affecting these ecosystems, the functional gene ammonia-monooxygenase  
22 was included in the quantitative analysis. Both bacteria (AOB) and archaea (AOA) ammonia-  
23 oxidisers drive the first and rate-limiting step of nitrification. Their use as process indicators  
24 provides important information due both to their function and to differential response to envi-  
25 ronmental factors influenced by their diverse ecological niches (Prosser and Nicol 2012).

26 DNA-targeting techniques do not discriminate the active from the total population and  
27 [the presence of highly resilient extracellular DNA and DNA deriving from dead cells may](#)  
28 [lead to a biased view of the ecosystem ecology \(Pietramellara et al. 2009\). Moreover, PCR-](#)  
29 [DGGE approach allows to perform a comparison among samples at “low resolution”,](#)  
30 [compared to metagenomic sequencing. However, the objective of this work was to investigate](#)  
31 [the long term influence of cryoturbation and edaphic properties on soil microbial community](#)  
32 [shaping. In this sense, the data obtained provided exhaustive information about the overall](#)

1 microbial complexity, the more represented groups and evidenced the ecosystem properties  
2 possibly involved in shaping and influencing the community.  
3 Overall, the composition of bacterial communities found on these patterned ground  
4 features results quite similar to those described [more in](#) detail on alpine soils (Nemergut  
5 et al. 2005), or in other periglacial landscapes like polygonal soils (Frank-Fahle et al. 2014),  
6 ice wedges (Wilhelm et al. 2012), or glacier forefields (Bajerski and Wagner 2013), in  
7 accordance with Delmont et al. (2014) that showed how similar habitats may lead to the  
8 development of communities with similar composition.  
9 The presence of phylotypes belonging to at least seven different phyla indicates quite  
10 complex bacterial communities. Among primary producers, phototrophic Cyanobacteria and  
11 Alphaproteobacteria families including chemolithotrophic and chemoorganotrophic organisms  
12 (Bradyrhizobiaceae, Rhodospirillaceae, Hyphomicrobiaceae) were detected. Moreover, in all  
13 the sites several Acidobacteria-related phylotypes were found. The ability to grow at low  
14 nutrient conditions and tolerate variations in soil humidity often characterize Acidobacteria  
15 (Ward et al. 2009), giving a potential explanation for their ubiquity in the examined patterned  
16 ground ecosystems. Finally, the presence of at least one representative of Bacteroidetes in all  
17 the sites suggests that these communities can host also a group of degraders of complex  
18 substrates (Nemergut et al. 2005).  
19 Archaea showed lower differentiation, with all the investigated phylotypes belonging  
20 to Thaumarchaeota division. Considering that Thaumarchaeota includes all known archaeal  
21 ammonia oxidizers, this result is also consistent with the high abundance of *amoA* gene  
22 copies, which exceed their bacterial analogues in all the samples. Similar situations are quite  
23 common in different ecosystems, particularly in acidic soils (Prosser and Nicol 2012; Qin et  
24 al. 2013; Tian et al 2014; Xu et al. 2012), and has been reported by Frank-Fahle et al. (2014)  
25 for polygonal tundra. Nevertheless, the same study pointed out a clear predominance of  
26 methanogens, not detected in this study. Previous studies highlighted the importance of  
27 waterlogging, common phenomenon affecting polygonal soils, in driving permafrost  
28 microbial community [composition](#) [structure](#) (Ollivier et al. 2014). However, the study sites  
29 present quite different characteristics, in terms of water content, 12-28% (unpublished data),  
30 from those reported for high latitude patterned ground ecosystems. Therefore, the  
31 predominance of aerobic phylotypes over anaerobic is not surprising and could indicate a  
32 relevant role of Thaumarchaeota in influencing [Nitrogen](#) availability in mid-latitude PGs.

1 In terms of archaeal sequences, the presence of identical sequences (100% similarity)  
2 in our samples and in both cold or rock-associated ecosystems and temperate agricultural and  
3 forest soils might suggest the cosmopolitan nature of at least a part of the community.  
4 In general, analysis of bacterial, archaeal and fungal phylogenetic markers revealed a  
5 quite homogeneous community composition within single PGFs, without a clear separation  
6 between samples collected from the vegetated rims and the central, nearly bare soil portion.  
7 This is also coherent with the results that Timling et al. (2014) obtained for fungal  
8 phylogenetic markers investigating patterned grounds in the northernmost bioclimatic  
9 subzone of the North American Arctic Transect.  
10 Differences in community composition were related more consistently to the sampling  
11 site than to the position across the PGFs. Also the NMDS analysis (Fig. 5), performed on the  
12 average band distribution of all the phylogenetic markers, supported this separation among  
13 sites, pointing out the main parameters (data from D'Amico et al. 2015) involved in site  
14 differentiation and, potentially, in shaping the composition of microbial community (Table 6).  
15 For instance, the GN site was located at the highest altitude, and was characterized by highest  
16 P content and exchangeable Ca/Mg ratio. Conversely, high levels of exchangeable Mg and Ni  
17 fitted with the SP community; CS sites were mainly characterized by high levels of Ca, while  
18 GB communities were correlated with intermediate levels of most soil parameters. This seems  
19 to suggest that in this mid-latitude, alpine context parent material lithology can be a strong  
20 driver for microbial community differentiation in terms of community composition,  
21 overcoming the effect of strong, small-scale gradients in edaphic properties produced by  
22 cryoturbation and patterned ground development. The importance of parent material lithology  
23 on composition of microbial communities has been described in a variety of different  
24 ecosystems, like soils (Reith et al. 2015), continental crystalline crust (Nyyssönen et al. 2014),  
25 pristine aquifers (Boyd et al. 2007) and arctic streams (Larouche et al. 2012). D'Amico et al.  
26 (2015), analysing the same study sites, reported a similar vegetation diversity pattern, with a  
27 lack of differentiation between rims and centres, and a strong separation of plant communities  
28 developed on different matrixes. Therefore, parent material lithology and the associated soil  
29 chemical properties, plant colonization and microbial community composition seem to be  
30 strictly linked.  
31 Quantitative analysis, performed by qPCR, presents a different picture. In fact, mi-  
32 crobial abundance resulted quite homogeneous among different sites. The only one showing

1 significantly lower abundances (in terms of Bacteria, Archaea, AOA markers and DNA) was  
 2 GN site. This is probably linked to the lower temperatures, associated to highest altitude,  
 3 which affects also total vegetation cover (D'Amico et al. 2015).

4 Conversely, some interesting patterns appear by comparing samples within single  
 5 features. In particular, concentric trends reported for bacterial, archaeal and fungal markers, as  
 6 well as for total DNA concentration, on sites CS, GB and GN are coherent not only with veg-  
 7 etation cover, but also with the small-scale variation of chemical properties, organic C and nu-  
 8 trient content (D'Amico et al. 2015). For instance, TOC, N and exchangeable bases decreased  
 9 from the rims to the centres, in parallel with microbial abundances, while pH, possibly also  
 10 affecting microbial activity, showed an opposite trend. This distribution has already been re-  
 11 ported for Arctic patterned grounds (González et al. 2014; Timling et al. 2014), where higher  
 12 levels of microbial biomass were found in vegetated rims if compared with patterned ground  
 13 features. However, one of the four sites did not follow this scheme. In fact, archaeal, bacterial  
 14 and total DNA abundances showed a different trend in SP site, increasing -or remaining  
 15 nearly constant, for bacteria- from the rims toward the centre of the sorted stripes, despite an  
 16 opposite trend of plant cover and contents of organic C and nutrients. SP site strongly differs  
 17 from the others for the high exchangeable Ni concentration, which increases from the centre  
 18 toward the rims. Therefore, in this case, it is possible that Ni toxicity becomes the prevalent  
 19 driving factor in microbial distribution across the features, overwhelming the effect of other  
 20 chemical properties. An inverse correlation between microbial biomass and respiration and Ni  
 21 content has been observed in subalpine forest soils in the same area by D'Amico et al. (2009).

22 Finally, exploring more in detail the relationships existing between microbial abun-  
 23 dances and soil chemical parameters (Table 7), Bacteria showed the highest number of signifi-  
 24 cant correlations with different chemical properties (vegetation cover and contents of Ca, Mg,  
 25 K, TN and TOC and C/N). On the other hand, Archaea abundances only correlated with the  
 26 soil C/N, but correlated to all the microbial markers except for bacterial *amoA*. Similar situa-  
 27 tions have been previously reported for alpine forest soils (Siles and Margesin 2016) and for  
 28 tundra soils (Blaud et al. 2015), with bacterial abundance following chemical soil properties  
 29 trend, and archaeal abundance independent from them. However, in those cases, all the micro-  
 30 bial markers resulted positively correlated to each other, suggesting an indirect action of envi-  
 31 ronmental parameters on the whole microbial population. In our case we can hypothesize that,

1 despite the presence of a bacterial population apparently more sensitive to variations in sub-  
 2 strate composition, Archaea seems to represent the link among the different microbial do-  
 3 mains, and so the real keystone of the ecosystem. Moreover, considering that Thaumarchaeota  
 4 seem to represent an important portion of archaeal community in this ecosystem, the low level  
 5 of correlation with any environmental parameter but C/N, reported for both archaeal and AOA  
 6 markers, could be linked to the wide ecophysiological potential of this group, including not  
 7 only autotrophy but also mixotrophy and heterotrophy lifestyles (Pester et al. 2011, Prosser  
 8 and Nicol 2012). Concerning fungal abundances, the only correlation with soil properties was  
 9 found with C/N. Nevertheless, the presence of a unique intra-feature distribution in gene-  
 10 abundance repeated in all the sites suggests the presence of other factors, not considered here  
 11 but suitable for further investigations, driving fungal distribution, such as organic matter com-  
 12 position and quality.

### 14 Conclusions

15 With this work, we obtained information about the overall complexity of the community and  
 16 the more represented microbial groups, giving a preliminary insight in a previously  
 17 unexplored ecosystem like alpine PG. Our results seem to indicate that Archaea and, in  
 18 particular, Thaumarchaeota seem to play a key role in ecosystem coordination end  
 19 functioning, suggesting this domain as a target for further, more detailed investigations.

20 In terms of ecological drivers, if micro-topographic heterogeneity produced by  
 21 cryogenic processes seems to influence microbial distribution within PG features in terms of  
 22 abundance, it has no clear effects on community composition. Conversely, lithology might  
 23 strongly influence community composition but has not evident effect on overall microbial  
 24 abundance, which is probably more linked to other variables, like altitude and temperature  
 25 conditions. Only in the serpentine sampling site it is possible to hypothesize an indirect  
 26 influence of lithology on small-scale microbial abundance distribution: in fact, the presence of  
 27 a gradient in heavy metals, produced by cryogenic processes, affects microbial distribution  
 28 determining opposite trends with respect to all the other parent materials.

29 In conclusion, our results offer a picture quite in accordance with previous studies focused on  
 30 Arctic PGFs, adding lithology to the complex hierarchy of controls modulating the effect of  
 31 cryoturbation on soil microbial communities.



Further studies are needed in order to assess how the investigated drivers impact on community diversity and its potential metabolic activity. Moreover, an RNA-based analysis would allow to compare not only spatial, but also seasonal or daily community variations, giving more insights on the real ecosystem functioning in relation to temperatures variation and exposure to freeze-thaw activity.

## References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic Local Alignment Search Tool. *J Mol Biol* 215:403–410
- Bajerski F, Wagner D (2013) Bacterial succession in Antarctic soils of two glacier forefields on Larsemann Hills, East Antarctica. *FEMS Microbiol Ecol* 85:128–142. doi: 10.1111/1574-6941.12105
- Ballantyne CK (2013) Patterned ground. In: Elias SA, Mock CJ (Eds) *The Encyclopedia of Quaternary Science*, 2nd edn. Elsevier, Amsterdam. pp 452–463
- Barrett JE, Virginia RA, Wall DH, Pearsons AN, Powers LE, Burkins MB (2004) Variation in biogeochemistry and soil biodiversity across spatial scales in a polar desert ecosystem. *Ecology* 85(11):3105–3118
- Blaud A, Phoenix GK, Osborn AM (2015) Variation in bacterial, archaeal and fungal community structure and abundance in High Arctic tundra soil. *Polar Biol* 38:1009–1024. doi: 10.1007/s00300-015-1661-8
- Bockheim JG, Munroe JS (2014) Organic carbon pools and genesis of alpine soils with permafrost: a review. *Arct Antarct Alp Res* 46:987–1006
- Boyd ES, Cummings DE, Geesey GG (2007) Mineralogy influences structure and diversity of bacterial communities associated with geological substrata in a pristine aquifer. *Microb Ecol* 54:170–182. doi: 10.1007/s00248-006-9187-9
- Cocolin L, Bisson LF, Mills DA (2000) Direct profiling of the yeast dynamics in wine fermentations. *FEMS Microbiol Lett* 189:81–87. doi: 10.1016/S0378-1097(00)00257-3
- D’Amico M (2009) Soil ecology and pedogenesis on ophiolitic materials in the western Alps (Mont Avic Natural Park, North-western Italy): soil properties and their relationships with substrate, vegetation and biological activity. Dissertation, Università degli Studi di Milano Bicocca
- D’Amico M, Gorra R, Freppaz M (2015) Small-scale variability of soil properties and soil-vegetation relationships in patterned ground on different lithologies (NW Italian Alps). *Catena* 135:47–58. doi: 10.1016/j.catena.2015.07.005
- Delmont TO, Francioli D, Jacquesson S, Laoudi S, Mathieu A, Nesme J, Ceccherini MT, Nannipieri P, Simonet P, Vogel TM (2014) Microbial community development and unseen diversity recovery in inoculated sterile soil. *Biol Fertil Soils* 50:1069–1076. doi: 10.1007/s00374-014-0925-8
- Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proc Natl Acad Sci USA* 102:14683–14688. doi: 10.1073/pnas.0506625102

1 Frank-Fahle BA, Yergeau É, Greer CW, Lantuit H, Wagner D (2014) Microbial functional po-  
2 tential and community composition in permafrost-affected soils of the NW Canadian  
3 Arctic. *PLoS ONE* 9(1): e84761. doi: 10.1371/journal.pone.0084761

4 González G, Rivera-Figueroa FI, Gould WA, Cantrell SA, Pérez-Jiménez JR (2014) Microor-  
5 ganisms in small patterned ground features and adjacent vegetated soils along topo-  
6 graphic and climatic gradients in the High Arctic, Canada. *Open J Soil Sci* 4:47–55

7 Jansson JK, Tag N (2014) The microbial ecology of permafrost. *Nat Rev Microbiol* 12:414–  
8 425. doi: 10.1038/nrmicro3262

9 Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (Eds) *Nucleic*  
10 *acid techniques in bacterial systematics*. Wiley, New York, pp 115–175

11 Larouche JR, Bowden WB, Giordano R, Flinn MB, Crump BC (2012) Microbial biogeogra-  
12 phy of arctic streams: exploring influences of lithology and habitat. *Front Microbiol* 3:1–  
13 9. doi: 10.3389/fmicb.2012.00309

14 Lawley B, Ripley S, Bridge P, Convey P (2004) Molecular analysis of geographic patterns of  
15 eukaryotic diversity in Antarctic soils. *Appl Environ Microbiol* 70:5963–5972. doi:  
16 10.1128/AEM.70.10.5963

17 Margesin R, Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. *Res*  
18 *Microbiol* 162:346–361. doi: 10.1016/j.resmic.2010.12.004

19 McTavish H, Fuchs JA, Hooper AB (1993) Sequence of the gene coding for ammonia  
20 monooxygenase in *Nitrosomonas europaea*. *J Bacteriol* 175:2436–2444

21 Michaelson GJ, Ping CL, Epstein H, Kimble JM, Walker DA (2008) Soils and frost boil eco-  
22 systems across the North American Arctic Transect. *J Geophys Res* 113:G03S11. doi:  
23 10.1029/2007JG000672

24 Michaelson GJ, Ping CL, Walker DA (2012) Soils associated with biotic activity on frost boils  
25 in Arctic Alaska. *Soil Sci Soc Am J* 76:2265–2277. doi: 10.2136/sssaj2012.0064

26 Muyzer G, Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by  
27 denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified  
28 genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695–700

29 Muyzer G, Teske A, Wirsén CO, Jannasch HW (1995) Phylogenetic relationships of Thiomicro-  
30 spirita species and their identification in deep-sea hydrothermal vent samples by dena-  
31 turing gradient gel electrophoresis of 16S rDNA fragments. *Arch Microbiol* 164:165–172

32 Nemerugut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SK  
33 (2006) Microbial community succession in an unvegetated, recently deglaciated soil. *Mi-  
34 crob Ecol* 53:110–122. doi: 10.1007/s00248-006-9144-7

1 Nemerugut DR, Costello EK, Meyer AF, Pescador MY (2005) Structure and function of alpine  
2 and arctic soil microbial communities. *Res Microbiol* 156:775–784. doi:  
3 10.1016/j.resmic.2005.03.004

4 Nicol GV, Glover LA, Prosser JI (2003) The impact of grassland management on archaeal  
5 community structure in upland pasture rhizosphere soil. *Environ Microbiol* 5(3):152–162

6 Nyssönen M, Hultman J, Ahonen L, Kukkonen I, Paulin L, Laine P, Itävaara M, Auvinen P  
7 (2014) Taxonomically and functionally diverse microbial communities in deep crystal-  
8 line rocks of the Fennoscandian shield. *ISME J* 8:126–138. doi: 10.1038/ismej.2013.125

9 O'Donnell K (1993) *Fusarium and its near relatives*. In: Reynolds DR, Taylor JW (Eds) *The*  
10 *fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics*.  
11 CAB International, Wallingford, UK, pp 225–233

12 Oksanen J, Guillaume Blanchet F, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson  
13 GK, Solymos MP, Stevens HH, Wagner H (2013) *Vegan: community ecology package*. R  
14 package version 2.0-8. <http://CRAN.R-project.org/package=vegan>.

15 Ollivier J, Yang S, Dörfer C, Welzl G, Kühn P, Scholten T, Wagner D, Schlöter M (2014) Bac-  
16 terial community structure in soils of the Tibetan Plateau affected by discontinuous per-  
17 malfrost or seasonal freezing. *Biol Fertil Soils* 50:555–559. doi: 10.1007/s00374-013-  
18 0869-4

19 O'Sullivan LA, Webster G, Fry JC, Parkes RJ, Weightman AJ (2008) Modified linker-PCR  
20 primers facilitate complete sequencing of DGGE DNA fragments. *J Microbiol Methods*  
21 75:579–581. doi: 10.1016/j.mimet.2008.08.006

22 Øvreås L, Forney L, Daae FL, Torsvik V (1997) Distribution of bacterioplankton in meromictic  
23 lake Sælenvannet, as determined by denaturing gradient gel electrophoresis of PCR-  
24 amplified gene fragments coding for 16S rRNA. *Appl Environ Microbiol* 63:3367–3373.  
25 doi: 0099-2240/97/\$04.0010

26 Pester M, Schleper C, Wagner M (2011) The Thaumarchaeota: an emerging view of their phy-  
27 logeny and ecophysiology. *Curr Opin Microbiol* 14:300–306. doi:  
28 10.1016/j.mib.2011.04.007

29 Pietramellara G, Ascher J, Borgogni F, Ceccherini MT, Guerri G, Nannipieri P (2009)  
30 *Extracellular DNA in soil and sediment: fate and ecological relevance*. [Biol Fertil Soils](http://www.sciencedirect.com/science/article/pii/S0926641009000458)  
31 45:219–235. doi: 10.1007/s00374-008-0345-8

32 Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for  
33 niche specialisation and differentiation. *Trends Microbiol* 20:523–531. doi:  
34 10.1016/j.tim.2012.08.001

35 Qin H, Yuan H, Zhang H, Zhu Y, Yin C, Tan Z, Wu J, Wei W (2013) Ammonia-oxidizing ar-  
36 chaea are more important than ammonia-oxidizing bacteria in nitrification and NO<sub>3</sub>–N

1 loss in acidic soil of sloped land. Biol Fertil Soils 49:767–776. doi: 10.1007/s00374-012-  
2 0767-1

3  
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61  
62  
63  
64  
65

3 Reith F, Zammit CM, Pohrib R, Gregg AL, Wakelin SA (2015) Geogenic factors as drivers of  
4 microbial community diversity in soils overlying polymetallic deposits. Appl Environ  
5 Microbiol 81:7822–7832. doi: 10.1128/AEM.01856-15

6  
7  
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57  
58  
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60  
61  
62  
63  
64  
65

6 Reyensbach AL, Pace NR (1995) Thermophiles. In: Robb FR, Place AR (eds) *Archaea: A La-*  
7 *boratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. pp 101–  
8 107

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10  
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61  
62  
63  
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65

9 Siles JA, Margesin R (2016) Abundance and diversity of bacterial, archaeal, and fungal com-  
10 munities along an altitudinal gradient in alpine forest soils: what are the driving factors?  
11 Microb Ecol. doi: 10.1007/s00248-016-0748-2

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60  
61  
62  
63  
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65

12 Steven B, Léveillé R, Pollard WH, Whyte LG (2006) Microbial ecology and biodiversity in  
13 permafrost. Extremophiles 10:259–267. doi: 10.1007/s00792-006-0506-3

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16  
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18  
19  
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61  
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65

14 Tian XF, Hu HW, Ding Q, Song MH, Xu XL, Zheng Y, Guo LD (2014) Influence of nitrogen  
15 fertilization on soil ammonia oxidizer and denitrifier abundance, microbial biomass, and  
16 enzyme activities in an alpine meadow. Biol Fertil Soils 50:703–713. doi:  
17 10.1007/s00374-013-0889-0

18  
19  
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21  
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56  
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58  
59  
60  
61  
62  
63  
64  
65

18 Timling I, Walker DA, Nussbaum C, Lennon NJ, Taylor DL (2014) Rich and cold: diversity,  
19 distribution and drivers of fungal communities in patterned-ground ecosystems of the  
20 North American Arctic. Mol Ecol 23:3258–3272. doi: 10.1111/mec.12743

21  
22  
23  
24  
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26  
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60  
61  
62  
63  
64  
65

21 Vetrani C, Jannasch HW, MacGregor BJ, Stahl DA, Reyensbach AL (1999) Population struc-  
22 ture and phylogenetic characterization of marine benthic archaea in deep-sea sediments.  
23 Appl Environ Microbiol 65:4375–4384

24  
25  
26  
27  
28  
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61  
62  
63  
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65

24 Wagner D, Lipski A, Embacher A, Gattering A (2005) Methane fluxes in permafrost habitats  
25 of the Lena Delta: effects of microbial community structure and organic matter quality.  
26 Environ Microbiol 7(10):1582–1592. doi: 10.1111/j.1462-2920.2005.00849.x

27  
28  
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27 Walker DA, Epstein HE, Romanovsky VE, Ping CL, Michaelson GJ, Daanen RP, Shur Y, Pe-  
28 terson RA, Krantz WB, Reynolds MK, Gould WA, Gonzalez G, Nicolsky DJ, Vonlan-  
29 then CM, Kade AN, Kuss P, Kelley AM, Munger CA, Tarnocai CT, Matveyeva NV,  
30 Daniëls FJA (2008) Arctic patterned-ground ecosystems: a synthesis of field studies and  
31 models along a North American Arctic Transect. J Geophys Res 113:G03S01. doi:  
32 10.1029/2007JG000504

33  
34  
35  
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61  
62  
63  
64  
65

33 Walker DA, Kuss P, Epstein HE, Kade AN, Vonlanthen CM, Reynolds MK, Daniëls FJA  
34 (2011) Vegetation of zonal patterned-ground ecosystems along the North America Arctic  
35 bioclimate gradient. Appl Veg Sci 14:440–463. doi: 10.1111/j.1654-109X.2011.01149.x

1 Ward NL, Challacombe JF, Janssen PH et al (2009) Three genomes from the phylum *Acido-*  
2 *bacteria* provide insight into the lifestyles of these microorganisms in soils. Appl Envi-  
3 ron Microbiol 75:2046–2056. doi: 10.1128/AEM.02294-08

4  
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60  
61  
62  
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64  
65

4 Webster G, Parkes RJ, Cragg BA, Newberry CJ, Weightman AJ, Fry JC (2006) Prokaryotic  
5 community composition and biogeochemical processes in deep subseafloor sediments  
6 from the Peru Margin. FEMS Microbiol Ecol 38:65–85. doi: 10.1111/j.1574-  
7 6941.2006.00147.x

8  
9  
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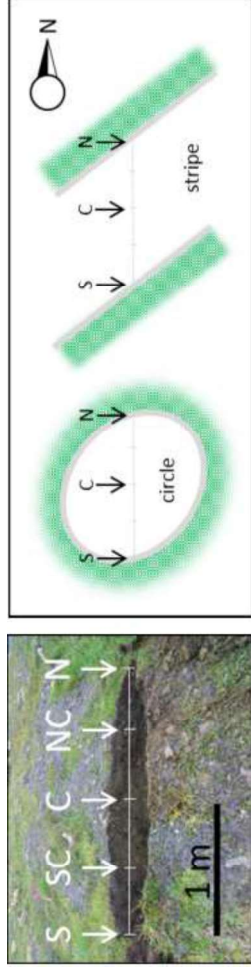
8 Wilhelm RC, Radtke KJ, Mykitezuk NCS, Greer CW, Whyte LG (2012) Life at the wedge:  
9 the activity and diversity of arctic ice wedge microbial communities. Astrobiology  
10 12:347–360. doi: 10.1089/ast.2011.0730

11  
12  
13  
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56  
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59  
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61  
62  
63  
64  
65

11 Xu YG, Yu WT, Ma Q, Zhou H (2012) Responses of bacterial and archaeal ammonia oxidis-  
12 ers of an acidic luvisols soil to different nitrogen fertilization rates after 9 years. Biol  
13 Fertil Soils 48:827–837. doi: 10.1007/s00374-012-0677-2

1 **Figure captions**  
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3 **Fig. 1** Sampling scheme on PG features. A north-south transect was drawn across the circles  
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5 (or the stripe, at site SP) and five surface samples (0-10 cm), equally spaced, were collected:  
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7 one central (C), two external (N and S), taken from the stony/vegetated rims and two  
8  
9 intermediate (NC and SC)  
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11 **Fig. 2** Cluster analysis of DGGE profiles obtained for bacterial, archaeal and fungal PG  
12  
13 communities (site 1=CS; 2=SP; 3=GB; 4=GN), based on Bray-Curtis dissimilarity algorithm  
14  
15 **Fig. 3** DNA concentration and abundance of bacterial and archaeal 16S rRNA genes, fungal  
16  
17 26S rRNA genes, archaeal and bacterial *amoA* genes in the four sites. Different letters indi-  
18  
19 cate significant differences ( $P < 0.05$ ) among sites according to Games Howell *post hoc* test  
20  
21 **Fig. 4** Distribution of different biological markers within single PG features in the four sites:  
22  
23 DNA concentration, bacterial and archaeal 16S rRNA genes, fungal 26S rRNA genes, ar-  
24  
25 chaeal and bacterial *amoA* genes  
26  
27 **Fig. 5** NMDS ordination of the four sites (1=CS; 2=SP; 3=GB; 4=GN), based on DGGE pro-  
28  
29 files (for each sampling point information obtained from archaeal, bacterial and fungal pro-  
30  
31 files were combined). Vectors show the direction and strength of environmental variables

Figure 1



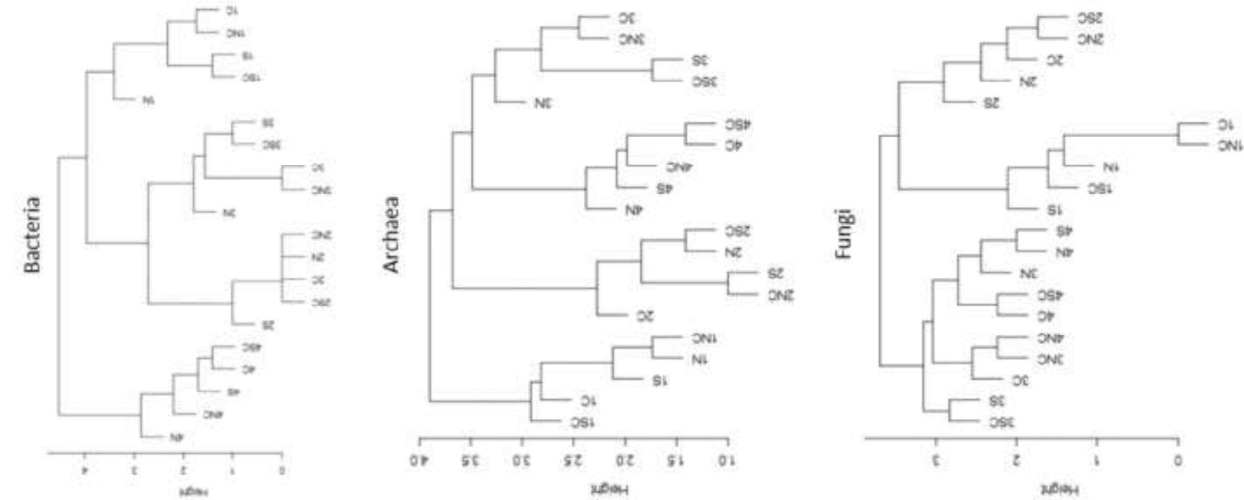
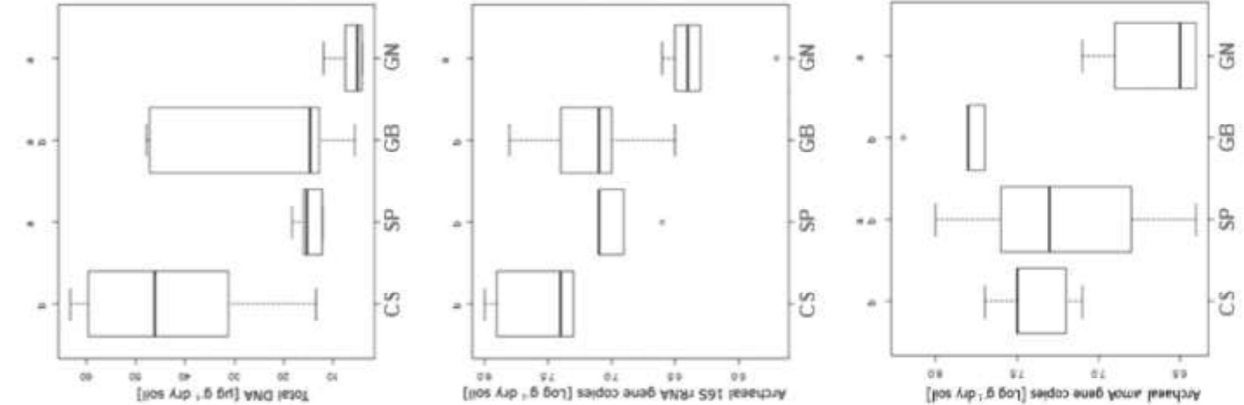


Figure 2

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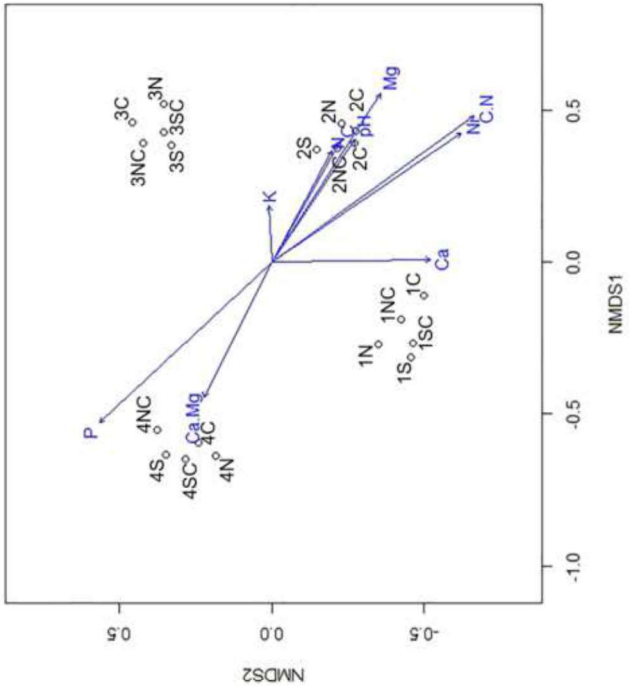


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Figure 5

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Figure 4

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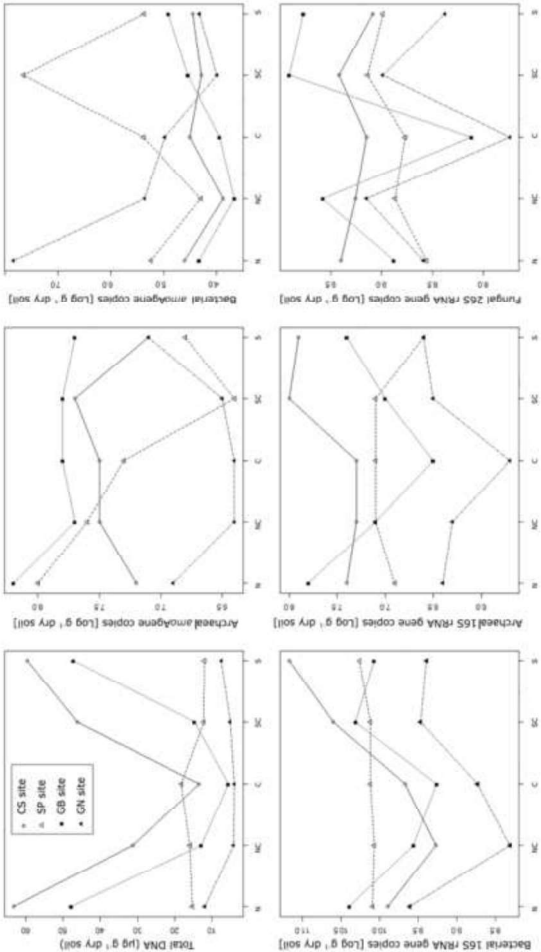


Table 1

1	2	Table 1 Localization and environmental properties of the study sites				
		Site localization	Coordinates	Elevation (m a.s.l.)	Parent material	PG type
3	4	1 - (CS)	Fenetre de Champorecher (Champorecher, AO)	2705	Calcschists (serpentinite in traces)	Nonsorted circles, hummocks
5	6					
7	8	2 - (SP)	Colle di Raye Chevrere (Champdepraz, AO)	2710	Serpentinite	Sorted stripes
9	10					
11	12	3 - (GB)	Lac des Heures (Champdepraz, AO)	2780	Gabbro (serpentinite in traces)	Sorted elongated circles
13	14					
15	16	4 - (GN)	Piata Lazin (Ronco Canavese, TO)	3054	Gneiss	Sorted circles
17	18					

2

Table 2

Site	Sample	pH	TOC (%)	C/N	Exchangeable Ca cmol kg <sup>-1</sup>	Exchangeable Mg cmol kg <sup>-1</sup>	Exchangeable Ni mg kg <sup>-1</sup>	Available P mg kg <sup>-1</sup>	Vascular plant cover (%)
CS	1N	5.2	2.65	14.7	6.32	2.05	11.77	8.09	98
	1NC	5.7	2.05	14.6	3.27	1.25	5.86	2.26	30
	1C	6	1.12	18.7	1.15	0.31	6.94	1.35	5
	1SC	5.3	3.01	14.3	3.76	1.23	3.50	2.66	20
SP	1S	5.5	3.12	14.2	6.57	1.18	1.94	7.18	100
	2N	5.7	1.59	13.3	1.30	1.64	24.74	2.41	50
	2NC	6	1.26	14.0	0.91	1.09	20.34	1.38	10
	2C	6.1	1.21	13.4	0.88	0.99	16.36	1.25	5
GB	2SC	5.9	3.33	15.1	1.31	1.97	20.12	2.26	20
	2S	5.4	11.78	13.4	5.02	4.57	30.24	10.77	50
	3N	5.4	4.21	16.8	2.92	1.52	0.03	12.02	40
	3NC	5.6	0.95	13.6	0.97	1.44	0.18	2.04	5
GN	3C	6.4	0.42	10.5	1.02	1.05	1.57	0.68	1
	3SC	5.3	2.43	12.2	1.15	0.53	0.00	6.08	10
	3S	5.2	6.16	15.0	2.58	1.99	1.34	12.71	30
	4N	5.3	0.60	12.0	0.24	0.07	0.00	14.3	5
4C	4NC	5.4	0.52	8.7	0.22	0.08	0.00	16.46	1
	4C	5.6	0.30	7.5	0.20	0.11	0.00	26.43	0
	4SC	5.4	0.42	8.4	0.26	0.08	0.00	14	1
	4S	5.3	0.27	6.8	2.06	0.06	0.00	9.77	5

Table 3

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Table 3 Closest 16S rRNA gene sequence matches to excised bacterial DGGE bands using the NCBI BLASTN search tool

DGGE band	Nearest match by BLASTN search (accession number)	Isolation environment of nearest sequence match	Sequence similarity (%)	Phylogenetic affiliation	Closest described species by BLASTN search (accession number)	Sequence similarity (%)	Presence in different PGs
							CS SP GB GN
B3	<i>Alphabacter aggregans</i> (NR_074324)	forest soil	95	<i>Acidobacteria</i>	<i>Alphabacter aggregans</i> (NR_074324)	95	x x x x
B8	<i>Nitrospira sibirica</i> (NR_074324)	-	100	<i>Alphaproteobacteria</i>	<i>Nitrospira sibirica</i> (NR_074324)	100	x x x x
B17	Uncultured bacterium clone KA13 (JQ973360)	forest soil	96	<i>Alphaproteobacteria</i>	<i>Micromonospora guangxiensis</i> (NR_044563)	95	x x x x
B5	Uncultured bacterium isolate DGGE gel band (L2 clone H1 (JX591052))	soil	99	<i>Alphaproteobacteria</i>	<i>Phaeosporium thuyum</i> (NR_023836)	94	x x x x
B2	Uncultured bacterium clone B18-80 (KF394065)	permafrost	99	<i>Alphaproteobacteria</i>	<i>Phaeosporium thuyum</i> (NR_023836)	97	x x x x
B7	Uncultured SOIL BACTERIUM clone G03AAZ03DP1 (JQ919759)	soil	96	<i>Bacteroidetes</i>	<i>Psychrobacter borealis</i> (NR_064381)	88	x x
B16	Chloroflex bacterium Ellin727 (AY673463)	soil	86	<i>Chloroflexi</i>	Chloroflex bacterium Ellin727 (AY673463)	82	x
B4	<i>Arthrobacter ramosus</i> (KF338765)	Himalaya	90	<i>Actinobacteria</i>	<i>Arthrobacter ramosus</i> (KF338765)	90	x
B15a	Uncultured bacterium clone (AB127883)	eutrophic lake	97	<i>Alphaproteobacteria</i>	<i>Stenotrophomonas anguise</i> (NR_044985)	95	x x
B24	Uncultured bacterium clone HPI27 (K833722)	soil	97	<i>Betaproteobacteria</i>	<i>Nitrospira multiformis</i> (NR_074736)	93	x
B15	<i>Crocococcus cyanobacterium</i> PFS6 (HE809540)	lake water	86	<i>Cyanobacteria</i>	<i>Cyanidium gracile</i> (NR_102447)	85	x
B8, B9	<i>Pseudomonas anarctica</i> (NR_025586)	McMurdo Valley, Antarctica	100	<i>Gammaproteobacteria</i>	<i>Pseudomonas anarctica</i> (NR_025586)	100	x
B12, B13	Uncultured bacterium clone periphytic soil (Himalaya)	periphytic soil	100	<i>Verrucomicrobia</i>	<i>Prothothoecephala vialla</i> (NR_023836)	86	x
B14	Uncultured bacterium isolate 111260339975 (BQ1871)	forest soil	95	<i>Verrucomicrobia</i>	<i>Bacterium Ellin807</i> (AY960770)	90	x

Table 4

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Table 4 Closest 16S rRNA gene sequence matches to excised archaeal DGGE bands using the NCBI BLASTN search tool

DGGE band	Nearest match by BLASTN search (accession number)	Isolation environment of nearest sequence match	Sequence similarity (%)	Phylogenetic affiliation	Closest described species by BLASTN search (accession number)	Sequence similarity (%)	Presence in different PGs
							CS SP GB GN
1	Uncultured archaeon clone DT-11-10 (KJ534140)	permafrost sediments (Olan maudun)	98	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	94	x x x x
A	Uncultured archaeon clone (KF444583)	Western Himalaya	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera evergladenensis</i> SRI (CF080774)	94	x x x x
B	Uncultured archaeon clone AS.A17 (G1298213)	glacial cryonite (Sury Island, Antarctica)	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	96	x x x x
C	Uncultured clone DT-14Q-2T-17 (KJ066470)	permafrost soil (Oughlun, Tibetian Plateau)	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	95	x x x x
D	Uncultured archaeon clone DZ2-14Q-2T-17 (KF466484)	permafrost soil (Oughlun, Tibetian Plateau)	100	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	95	x x x x
E	Uncultured archaeon clone ABCde-50 (GQ26886)	soil (Mount Mita, Tibetian Plateau)	97	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	93	x x x x
68	Uncultured archaeon clone ABCde227 (GQ127550)	soil (Mount Mita, Tibetian Plateau)	96	<i>Thaumarchaeota</i>	<i>Candidatus Nitrososphaera gargensis</i> G09.2 (NR_102916.1)	94	x

Table 6

Table 6 Correlation values and significance between the soil chemical properties and the NMDS factors shown in Fig.5

	NMDS1	NMDS2	r <sup>2</sup>	P-Value
pH	0.83	-0.56	0.21	0.118
Ca	0.02	-1.00	0.25	0.089
Mg	0.84	-0.54	0.40	0.008
K	1.00	0.04	0.03	0.756
Ni	0.56	-0.83	0.52	0.008
Ca/Mg	-0.89	0.45	0.22	0.003
N	0.88	-0.48	0.16	0.222
C	0.87	-0.50	0.19	0.140
C/N	0.59	-0.81	0.61	0.001
P	-0.68	0.73	0.54	0.001
Altitude	-0.65	0.76	0.91	0.001

Table 5

Table 5 Closest 26S rRNA gene sequence matches to excised fungal DGGE bands using the NCBI BLASTN search tool

DGGE band	Nearest match by BLASTN search (accession number)	Isolation environment of nearest sequence match	Sequence similarity (%)	Phylogenetic affiliation	Closest described species by BLASTN search (accession number)	Sequence similarity (%)	Presence in different PCs			
							CS	SP	GB	GN
F1	Uncultured fungus clone 126, NA10 PF3, C19 (KC966197)	Petwood Ground (North American Ascle)	98	<i>Ascomycota</i>	<i>Gaeumannomyces</i> sp. (AB752287)	97	x	x	x	
F2	<i>Phytophthora</i> (JX244063)	<i>Populus deltoides</i> roots	95	<i>Ascomycota</i>	<i>Phytophthora</i> sp. (JX244063)	95	x	x	x	
F3, F4, F5	Uncultured fungus clone 112, NA3 PF1, PT1 (KC966078)	Petwood Ground (North American Ascle)	97	<i>Ascomycota</i>	<i>Gaeumannomyces</i> sp. (AB752287)	97	x	x	x	x
F6	<i>Ceratomyces</i> cf. <i>variosus</i> (F8687648)	alpine soil (root apex of <i>Salix herbacea</i> )	97	<i>Basidiomycota</i>	<i>Ceratomyces</i> cf. <i>variosus</i> (F8687648)	97	x	x		
F7	<i>Gleadowia</i> cf. <i>crinita</i> (JX244063)	Guillem Massif	89	<i>Basidiomycota</i>	<i>Gleadowia</i> cf. <i>crinita</i> (JX244063)	89			x	x
F8	Uncultured fungus clone 126, NA10 PF3, C19 (KC966197)	Petwood Ground (North American Ascle)	98	<i>Ascomycota</i>	<i>Gaeumannomyces</i> sp. (AB752287)	97	x	x	x	
F2	<i>Phytophthora</i> (JX244063)	<i>Populus deltoides</i> roots	95	<i>Ascomycota</i>	<i>Phytophthora</i> sp. (JX244063)	95	x	x	x	

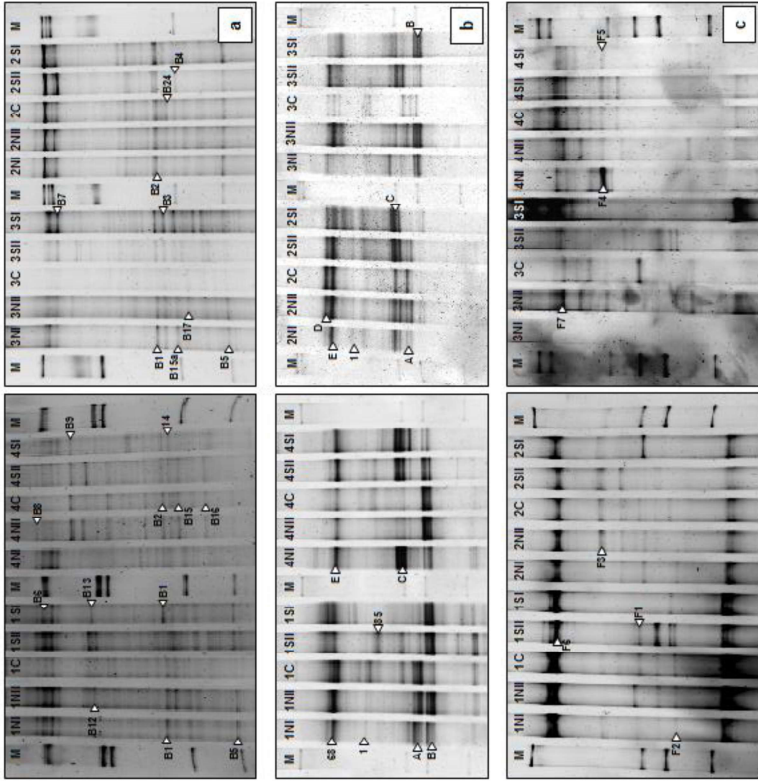
Variables	Bact	Arch	Fun	AOB	AOA	DNA
Plant coverage %	0.698**	0.666**	0.321	0.094	0.281	0.802**
pH	-0.167	-0.129	-0.396	-0.089	0.047	-0.187
Ca	0.588**	0.717**	0.368	-0.164	0.257	0.710**
Mg	0.478*	0.534*	0.362	0.060	0.275	0.594**
K	0.606**	0.349	0.343	0.224	-0.108	0.414
Ni	0.412	0.352	0.009	0.163	0.123	0.430
Cu/Mg	-0.089	0.075	0.071	-0.144	-0.230	0.024
P	-0.162	-0.347	-0.620	0.326	-0.439	-0.264
N	0.789**	0.643**	0.526*	0.186	0.216	0.695**
C	0.785**	0.654**	0.492*	0.205	0.229	0.712**
C/N	0.510*	0.815**	0.526*	-0.033	0.287	0.747**
Bact		0.626**	0.284	0.185	0.329	0.672**
Arch			0.556*	-0.323	0.480*	0.916**
Fun				-0.195	0.197	0.454*
AOB					-0.450*	-0.125
AOA						0.495*

\*p < 0.05, \*\*p < 0.01,

Online Resource 1

Comparison among sites and sampling points

DGGE profiles of bacterial 16S rRNA genes (a), archaeal 16S rRNA genes (b) and fungal 26S rRNA genes (c) in the four sites



(Biology and Fertility of Soils)

Driving factors of soil microbial ecology in alpine, mid-latitude patterned grounds (NW Italian Alps)

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Online Resource 3

Primer sets and amplification details used for qPCR analysis

Primer pair	Sequence (5'-3') <sup>a</sup>	Target gene	Fragment length	Reference	Amplification details	Application
27F	AGA GTT TGA TCM TGG CTC AG	<i>Bacteria</i> 16S rRNA	c. 1500	Lane 1991	qPCR standard	qPCR standard
1492R	GGT TAC CTT GTT ACG ACT T					
S-D-Arch-0025-a-S-17	CTG GTT GAT CCT GCC AG	<i>Archaea</i> 16S rRNA	c. 1500	Verriani et al. 1999	qPCR standard	qPCR standard
1517R						
	GCC ATA TCA ATA AGC GGA GGA AAA G	Fungal 26S rRNA	c. 250	OTDonnell 1993	95 °C 5 min; 44 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
NLI	ATT CCC AAA CAA CTC GAC TC			Coccolin et al. 2000		
LS2				Mc Tavish et al. 1993	94 °C 3 min; 40 cycles: 95 °C 30 s, 55 °C 30 s, 72 °C 45 s	qPCR standard, qPCR
amoA-1F	GGG GTT TCT ACT GGT GGT	Bacterial <i>amoA</i> gene	491			
amoA-2R	CCC CTC KGS AAA GCC TTC TTC					
Arch-amoAF	STA ATG GTC TGG CTT AGA CG	Archaeal <i>amoA</i> gene	635	Francis et al. 2005	95 °C 30s; 40 cycles: 95 °C 30 s, 50 °C 30 s, 72 °C 1 min	qPCR standard, qPCR
Arch-amoAR	GCG GCC ATC CAT CTG TAT GT					
519F	CCA GCA GCC GCG GTA AT AC	<i>Bacteria</i> 16S rRNA	c. 390	Lane 1991	95 °C 30 s; 40 cycles: 95 °C 30 s, 50 °C 30 s, 72 °C 1 min	qPCR
907R	CCG TCA ATT CMT TTR AGT TT			Muyzer et al. 1995		
S-D-Arch-0025-a-S-17	CTG GTT GAT CCT GCC AG	<i>Archaea</i> 16S rRNA	c. 320	Verriani et al. 1999	95 °C 30 s; 40 cycles: 95 °C 30 s, 48 °C 30 s, 72 °C 1 min	qPCR
S-D-Arch-0344-a-S-20	TCG CGC CTG CTG CGC CCC GT					

<sup>a</sup> D=G, A or T; H=A, T or C; K=G or T; M=A or C; R=A or G; S=G or C; W=A or T; Y=C or T

Biology and Fertility of soils

Driving factors of soil microbial ecology in alpine, mid-latitude patterned grounds (NW Italian Alps)

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